



PATENT
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10. A method for producing plants or parts thereof having an increased tolerance against drought or fungal infections or increased salt concentrations or extreme temperature (heat, cold), comprising:
 - (a) transfecting a plant, a plant tissue or a plant cell with a nucleic acid which encodes a (poly)peptide with an intrinsic affinity to plasmodesmata.
11. The method of claim 10, further comprising:
 - (b) regenerating a plant from the transfected plant cell.
12. The method of claim 11, further comprising:
 - (c) producing plants or plant cells from the plant regenerated in (b).
13. The method of any one of claims 10 to 12, wherein the (poly)peptide is a virus-encoded transport protein.
14. The method of claim 13, wherein the virus-encoded transport protein is the potato leaf roll virus-(PLRV) transport protein p17 or a derivative thereof.
15. The method of claim 14, wherein the derivative is a p17-protein with a hydrophilic N-terminal extension.
16. The method of claim 15, wherein the hydrophilic extension is the amino acid MAELGSGSELHRGGRSRTS (SEQ. ID. NO. 1).
17. The method of any one of claims 10 to 12, wherein the plant, the plant tissue or the plant cells are derived from potato, from tobacco, from cereals or vegetables or are potatoes, tobacco plants, cereal plants or vegetable plants.

18. The method of any one of claims 10 to 12, wherein the increase in tolerance of plants against fungal infections is an increase in tolerance against infections with *Phytophtora infestans*.
19. The method of claim 13, wherein the plant, the plant tissue or the plant cells are derived from potato, from tobacco, from cereals or vegetables or are potatoes, tobacco plants, cereal plants or vegetable plants.
20. The method of claim 14, wherein the plant, the plant tissue or the plant cells are derived from potato, from tobacco, from cereals or vegetables or are potatoes, tobacco plants, cereal plants or vegetable plants.
21. The method of claim 15, wherein the plant, the plant tissue or the plant cells are derived from potato, from tobacco, from cereals or vegetables or are potatoes, tobacco plants, cereal plants or vegetable plants.
22. The method of claim 16, wherein the plant, the plant tissue or the plant cells are derived from potato, from tobacco, from cereals or vegetables or are potatoes, tobacco plants, cereal plants or vegetable plants.
23. The method of any one of claims 13, wherein the increase in tolerance of plants against fungal infections is an increase in tolerance against infections with *Phytophtora infestans*.
24. The method of any one of claims 14, wherein the increase in tolerance of plants against fungal infections is an increase in tolerance against infections with *Phytophtora infestans*.
25. The method of any one of claims 15, wherein the increase in tolerance of plants against fungal infections is an increase in tolerance against infections with *Phytophtora infestans*.
26. The method of any one of claims 16, wherein the increase in tolerance of plants against fungal infections is an increase in tolerance against infections with *Phytophtora infestans*.
27. The method of any one of claims 17, wherein the increase in tolerance of plants against fungal infections is an increase in tolerance against infections with *Phytophtora infestans*.



broad-spectrum resistance against potato viruses PLRV, PVY and PVX as well as an increased concentration of sugar and sugar derivatives. For the expression in plants this gene was brought under transcriptional control of the 35S-promoter and —terminator of the cauliflower-mosaic-virus (CaMV) in the vector pRT1O3 (Topfer (1987), Nucleic Acids Res. 15: 5890) and this transcription unit (Figure 1) was then integrated into the binary plant transformation vector pBIN19 (Bevan (1984), Nucleic Acids Res. 12: 8711-8721). This vector was transferred to the *Agrobacterium tumefaciens* LBA4404 (pAL4404) (Hoekema (1983), Nature 303: 179-180) which was used for the transformation of *Solanum tuberosum* Var. Linda. Four (L4, L6, L7 and L3; see also Tacke (1996), op. cit.) of the independent

10 transgenic potato lines as well as the initial potato variety Linda were chosen for further tests concerning induced tolerance.

As already mentioned above the hydrophilic extension comprises the amino acid

Sequence MAELGSGSELHRGGGRSRTS (SEQ ID NO. 1) in a particularly preferred embodiment of the use according to the invention.

15 In another embodiment of the use according to the invention the plant, the plant tissue or the plant cells stem from the potato, from tobacco, from cereals or vegetables or are potatoes, tobacco plants, cereal plants or vegetable plants.

In a further preferred embodiment of the use according to the invention the increase in the tolerance of the plants against fungal infections is a tolerance against infections with
20 *Phytophthora infestans*.

As a surprising result according to this preferred embodiment it was found that transgenic lines received by the method according to the invention also distinguish themselves by a statistically significant tolerance against *Phytophthora infestans*, the pathogen of late blight of potato.

25 The invention further relates to the production of plants or parts thereof with an increased tolerance against drought and/or fungal infections and/or increased salt concentrations and/or extreme temperature (heat, cold), wherein

(a) a plant, a plant tissue or a plant cell is transfected with a nucleic acid coding for a (poly)peptide with an intrinsic affinity to plasmodesmata.

30 Additionally, in a preferred embodiment of the method according to the invention
(b) a plant is regenerated from the transfected plant cell.

In a particularly preferred embodiment of the method according to the invention

(c) further plants or plant cells are produced from the plant gained in (b) subsequent to step (b).

In a further preferred embodiment of the method according to the invention, the polypeptide is a virus-encoded transport protein.

5 In a particularly preferred embodiment of the method according to the invention the virus-encoded transport protein is the potato leaf roll virus-(PLRV) transport protein prl7 or a derivative thereof.

In a further preferred embodiment of the method according to the invention the derivative is a prl7 protein with a hydrophilic N-terminal-extension.

10 As already mentioned above in a particularly preferred embodiment of the method according to the invention the hydrophilic extension comprises the amino acid sequence MAELGSGSELHRGGRSRTS (SEQ ID NO. 1).

In another embodiment of the method according to the invention the plant, the plant tissue or the plant cells stem from potato, from tobacco, from cereals or vegetables or are potatoes,

15 tobacco plants, cereal plants or vegetable plants.

In a further preferred embodiment of the method according to the invention the increase in tolerance of the plant against fungal infections is a tolerance against infections with Phytophthora infestans.

The Figures show:

20 **Figure 1**

Production of the plasmid p17N. By specific mutagenesis the two AUG-codons of the wildtype prl7-gene were mutated to ACG and a translation initiation codon was inserted into the polylinker sequence.

Figure 2

25 Nucleotide and amino acid sequence of the mutated prl7N-gene or -protein MAELGSGSELHRGGRSRTS (SEQ ID NO. 1).

Figure 3

Result of the resistance test #1 (in the green-house) with 5 plants each of the initial variety Linda (L) as well as the prl7N-transgenic; lines L4, L6, L7 and L8.

30 A. Overall view of the test B. View of one plant each per line.

Figure 4

Result of the resistance test #2 (in the phytochamber) with 6 plants each of the initial type Linda (L) as well as of the pr17N-transgenic lines L4, L6, L7 and L8.

A. Partial view of the overall test. B. View of selected plants.

5 **Figure 5**

Bonitration of the infestation of leaf disks in the laboratory test with *P.infestans* race 1-11 (test #2) after 9, 10 and 13 days after the infection (dpi).

Figure 6

10 Cumulative depiction of two tests concerning the infection of potato leaf disks with *P.infestans* race 1-11.

Figure 7

Habitus of the non-transgenic potato variety Linda (L) and of the 4 transgenic lines L4, L6, L7 and L8 after 5 wee at 100 mM NaCl. Individual plants of the partial test (A) were described for a better depiction in (B). Concerning the initial variety Linda 15 the lower leaves died off.

Figure 8

Habitus of individual plants of the non-transgenic potato variety Linda (L) and of the 4 transgenic lines L4, L6, L7 and L8 after 5 weeks with 100. mM NaCl. Concerning variety Linda there can be still recognised residues of the died off lower leaves as well as 20 modifications of the stalk.

The Examples explain the invention.

Example 1: Production of the plasmid p17N

A modification at the 5-end of the pr17-gene (ORF4) was achieved by translational fusion of the multiple cloning site of the Bluescript-vector, insertion of an optimised translation 25 initiation codon as well as mutation of the two pr17-WT AUG initiation codon to ACG (Figure 1), Thi1 modification results in the expression of a derivative (pr17-N) of the pr17-WT-protein with a hydrphilic extension through the sequence
MAELGSGSELHRGGRGRSRTS (SEQ ID NO. 1) at the amino terminus (Tacke (1996), op. cit.; Figure 2). The production of the plasmid p17N is described in Schmitz (1996), Nucleic 30 Acids Res. 24: 257-263 (therein named p17/NIII).